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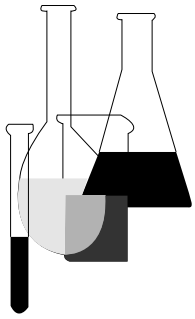
Ecological Effects Test Guidelines

OPPTS 850.4800
Plant Uptake and Translocation Test



"Public Draft"

INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Public Draft Access Information: This draft guideline is part of a series of related harmonized guidelines that need to be considered as a unit. *For copies:* These guidelines are available electronically from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines’’ or in paper by contacting the OPP Public Docket at (703) 305–5805 or by e-mail: guidelines@epamail.epa.gov.

To Submit Comments: Interested persons are invited to submit comments. By mail: Public Docket and Freedom of Information Section, Office of Pesticide Programs, Field Operations Division (7506C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person: bring to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Comments may also be submitted electronically by sending electronic mail (e-mail) to: guidelines@epamail.epa.gov.

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on *The Federal Bulletin Board*. By modem dial 202–512–1387, telnet and ftp: fedbbs.access.gpo.gov (IP 162.140.64.19), or call 202–512–0135 for disks or paper copies. This guideline is also available electronically in ASCII and PDF (portable document format) from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines.’’

OPPTS 850.4800 Plant uptake and translocation test.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline is 40 CFR 797.2850 Plant Uptake and Translocation Test.

(b) **Purpose.** The guideline in this section is intended for use in developing data on the uptake and translocation of chemical substances and mixtures (''chemicals'') by terrestrial plants subject to environmental effects test regulations. This guideline prescribes tests using commercially important terrestrial plants to develop data on the quantity of chemical substances incorporated in plant tissues and the potential for entry into food chains with resultant indirect human exposure. EPA will use data from these tests in assessing the hazard of a chemical to the environment.

(c) **Definitions.** The definitions in section 3 of the Toxic Substances Control Act (TSCA), and 40 CFR Part 792—Good Laboratory Practice Standards apply to this test guideline. The following definitions also apply to this guideline:

EC X means the experimentally derived chemical concentration that is calculated to effect X percent of the test criterion.

Mass balance means a quantitative accounting of the distributions of chemical in plant components, support medium, and test solutions. It also means a quantitative determination of uptake as the difference between the quantity of gas entering an exposure chamber, the quantity leaving the chamber, and the quantity adsorbed to the chamber walls.

Support media means the sand or glass beads used to support the plant.

Translocation means the transference or transport of chemical from the site of uptake to other plant components.

(d) **Test procedures**—(1) **Summary of the test—**(i) **Root exposure.** In preparation for the test, seeds are planted in the potting containers (or in cotton or glass-wool plugs supported in hydroponic solution) and, after germination, seedlings thinned, by pinching the stem at the support surface. Potting mixtures of sand or glass beads should be subirrigated with nutrient solution. Chemicals are applied to the plants via nutrient solution or adsorbed to the support media. Carrot, lettuce, onion, cabbage, and ryegrass may be harvested whenever there is adequate plant material for chemical analysis. Cucumber, corn, soybean, tomato, and oats should be grown until fruit or seed are mature.

(ii) **Foliar exposure.** The foliar exposure test is identical to the root exposure test except that chemicals are applied to plants by either spraying or dusting the foliage or exposing the plants to gas in a fumigation chamber. If plants are fumigated, either rates of uptake and surface adsorption should be calculated, or the plants may be harvested and analyzed for test chemical and residues.

(2) **Chemical application**—(i) **Root exposure.** (A) Chemicals that are soluble in water should be dissolved in the nutrient solution just prior to the beginning of the test. Deionized or glass-distilled water should be used in making stock solutions of the test chemical. Sufficient quantities of each concentration should be made up as needed to minimize storage time and disposal volume.

(B) Chemicals that are insoluble in water but which can be placed in aqueous suspension with a carrier should be added, with the carrier, to the nutrient solution. The carrier should be soluble in water, relatively nontoxic to plants, and should be used in the minimum amount required to dissolve or suspend the test chemical. There are no preferred carriers; however, acetone, gum arabic, polyethylene glycol, ethanol, and others have been used extensively in testing herbicides, plant growth regulators, fungicides, and other chemicals that affect plants. Carrier controls shoud be included in the experimental design and tested simultaneously.

(C) Water-insoluble chemicals for which no nontoxic, water-soluble carrier is available, should be dissolved in an appropriate volatile solvent. The solution should be mixed with the sand or glass beads which are then placed in a rotary vacuum apparatus and evaporated, leaving a uniform coating of chemical on the sand or beads. A weighed portion of beads should be extracted with the same organic solvent and the chemical assayed before the potting containers are filled. Solvent controls should be included in the experimental design and tested simultaneously.

(ii) **Foliar exposure.** (A) Water soluble chemicals should be dissolved in deionized or glass distilled water just prior to use. Sufficient quantities of each concentration should be made up as needed. These solutions should be applied at weekly intervals. Plants should be placed in an exhaust hood and the chemical applied to the foliage. A plastic sleeve may be fitted over the top of the pot, and the foliage sprayed with specific quantities of test solution at known concentrations. The plastic sleeve, confining the chemical to plant and pot, facilitates expression of chemical dosage as quantity per pot area (i.e., micrograms per square meter). Shoots of control plants should be sprayed in an identical manner with deionized or distilled water. Alternatively, a miniature compressed-air sprayer may be mounted on a pendulum and equipped to automatically spray a plant positioned directly beneath the center of its arc of swing. When radioisotope-labelled chemicals are applied, health and safety considerations pro-

hibit spray application. Instead, specific quantities of labelled chemical should be applied directly to leaves in single drops.

(B) Water-insoluble chemicals, existing as solids, may be prepared for testing by grinding or other reduction to particles of <200 μm diameter. These chemicals should be applied at weekly intervals. Plants should be placed in an exhaust hood, a plastic sleeve fitted over the top of the pot, and a specific quantity of chemical sprinkled uniformly over them. Prior to chemical application, plants should be misted with water to promote foliar retention of the chemical. Control plants also should be misted with deionized or distilled water at each treatment date and dusted with an inert material of the same particle size. Applications should be expressed as quantity per unit pot area (i.e., micrograms per square meter).

(C) Chemicals existing in gaseous form at normal ambient temperatures and pressures should be generated for use as needed or stored under pressure. The bottled gas may be 100 percent pure chemical or mixed with an inert carrier, such as nitrogen, to known concentrations. Chemicals of controlled or measured concentrations should be metered into the exposure chamber, uniformly mixed about the plants, and exhausted through the outlet port where the flow rate and concentration are again measured. Use of this systems design provides an alternate method of analysis if the quantity of chemical sorbed by plants is less than that required for chemical analysis. Plants should be fumigated whenever they have reached sufficient size for measurement of photosynthesis and transpiration rates, assuming equivalent detection sensitivity of carbon dioxide, water vapor, and chemical analyzers. The appropriate size is a function of the gas exchange system and constitutes an area of expert judgment.

(3) **Range-finding test.** (i) A range-finding test should be conducted to establish the chemical concentrations used in the uptake and translocation test.

(ii) Because of the different mechanisms involved in root and leaf uptake, and to more closely define the chemical concentrations to be used in the uptake test, the definitive early seedling growth test is recommended as the range-finding test. Seeds should be germinated directly in containers filled with sand or glass beads or in cotton or glass-wool plugs supported in hydroponic solution. When 50 percent of the seedlings have germinated, the seedlings should be thinned (by pinching) to the 10 most uniform per container and exposed to a concentration series of test chemical. The lowest concentration in the series, exclusive of controls, should be at or below the EC10 while the upper concentration should be at or above the EC90. If the anticipated fate of the chemical is soil or soil-water, and the mechanism of concern is root uptake, the chemical should be applied in nutrient solution to the root support media (or coated on sand or glass beads for non-water-soluble chemicals). With a chemical whose anticipated mode of exposure to plants is surface deposition by atmospheric transport or

irrigation water, the appropriate testing method may be foliar application allowing subsequent movement into the rooting zone with watering. Effect is assessed as growth reduction. The concentration selected as the upper limit for the uptake and translocation test should be near the threshold of visible injury. Short exposure periods to gas in fumigation chambers are not expected to promote visible injury or gross reductions in growth but may alter stomatal resistance, transpiration, or photosynthesis. Absorption and adsorption rates may be calculated and gas concentrations for definitive testing selected based on the calculated sorption rates.

(iii) Alternatively, the seed germination/root elongation test or other appropriate phytotoxicity test may be used to establish the appropriate upper concentration for testing.

(4) **Definitive test.** (i) The purpose of the uptake and translocation test is to determine the propensity for a chemical's accumulation in plants or plant parts.

(ii) At least three concentrations of chemical, exclusive of controls, should be used in the uptake test. Recommended concentrations would be a descending geometric progression from the upper concentration tested (i.e. 100, 50, 25 mg/L). A minimum of six replicate pots per concentration, each containing from one to four seedlings, should be used. If techniques other than radioistopes are used to determine uptake, more replicates may be required to provide sufficient plant materials for analysis. Test chemicals should be added to the hydroponic or nutrient solution or coated on glass beads for the root uptake test; or sprayed, dusted, or gassed directly on the foliage in the foliage uptake tests. Only untreated seed (not treated with fungicides, repellants, etc.) taken from the same lot, and year or season of collection should be used in a given test.

(iii) Control pots should be included in the experimental design and should be used in each run. In addition, a carrier control should be used for those chemicals that need to be solubilized.

(iv) If plants are to be grown hydroponically, seeds should be planted in plugs of cotton or glass-wool supported in the tops of the containers. When sand or glass beads are used, the recommended planting procedure is to fill potting containers with sand or glass beads to within 2.5 cm of the top and to sow seeds directly. After germination, the seedlings should be thinned by pinching the stem at the support surface. From one to four seedlings per potting container are required depending on species tested, the size of the containers, and the size to which the plants will grow. When plants are grown hydroponically, one plant per pot will probably be the preferred method. The number of plants selected should provide sufficient biomass for analytical procedures. A greater number of plants may be required depending on species tested, duration of test, and

analytical procedures. Too many plants in a container may actually reduce the growth and biomass.

(v) Alternate planting methods may be required when the chemical is highly volatile. An impervious barrier of polyethylene film, a modification of the double pot method, a glass plate, or other appropriate apparatus should be used to prevent volatilization from the root zone. Seeds should be germinated in the dark at 25 °C and seedlings with radicle length in the median range transplanted into the potting containers. The seedlings should be positioned such that their roots are exposed to the support media while the shoots pass through holes in the barrier. A ring of inert, non phytotoxic, pliable putty should be used to seal the holes around the stems. Control pots should be handled identically except there is to be no exposure to the test chemical. This transplanting procedure, without the volatilization barrier, is also recommended when the test chemical is adsorbed to the support medium.

(vi) Hydroponic solutions should be aerated and sand or glass filled potting containers should be periodically filled with nutrient solution and drained to provide aeration. For root exposure tests, the test chemical should be added to the nutrient solution or directly to substrate. The entire test solution should be replaced weekly, or earlier if the concentration of chemical in the test or nutrient solution varies by more than 20 percent of that specified. The volume of solution added should be recorded.

(vii) The test consists of one run for each of two specified plant species. The duration of a run, for solid and liquid chemicals, should be equal to the length of time required for the particular test variety to achieve sufficient biomass for testing. The duration of a run for gaseous chemicals should be the length of time required to make the specified gas exchange measurements. For a particular chemical, a run is defined as exposure of the plant species of three concentrations of test chemical with a minimum of six replicate pots and appropriate controls. Exposure is followed by extraction and analysis for parent compound, metabolites, and bound residues in plant tissues, and in the whole plants for solids, liquids, and gasses or by calculating rates of absorption and adsorption of gasses.

(viii) Visible effects (stunting of growth, discloration, chlorosis and/or necrosis of the leaves, decreased moisture content, or morphological abnormalities, etc.) should be recorded.

(ix) A randomized complete block design is recommended for this test, with blocks delineated within the chambers or over greenhouse benches and randomization of treatments occurring within the blocks. If, because of very large pots and plants, there exists inadequate space within chambers for blocking, total randomization within chambers is acceptable. This design is also appropriate for the growth of plants to be used for foliar exposure with gas.

(x) Irradiation measurements should be taken to the top of the plant canopy and the mean, plus a maximum and a minimum value, determined over the plant-growing area. These measurements should be taken at the start of the test, at biweekly intervals during the test, and at test termination. If the test is conducted in a greenhouse facility, hourly measurements of irradiation should be recorded and presented as daily total irradiance plus representative hourly curves for clear sky conditions and cloudy days.

(xi) Temperature and humidity measurements should be measured daily at the top of the plant canopy during each light and dark period.

(xii) Measurements of carbon dioxide concentration should be made at the top of the plant canopy (of chamber-growth plants) on a continuous basis.

(xiii) The amount of water and nutrient solution depleted each week should be recorded, to observe changes in evapotranspiration rates which may indicate stress. Furthermore, these data will be used to compute chemical uptake per volume of water transpired for the uptake test.

(5) **Analytical measurements**—(i) **Solid or liquid test chemicals.** (A) Stock solutions should be diluted with glass distilled or deionized water to obtain the test solutions. Standard analytical methods, if available, should be used to establish concentrations of these solutions and should be validated before beginning the test. An analytical method is not acceptable if likely degradation products of the chemical, such as hydrolysis and oxidation products, give positive or negative interference. The pH of these solutions should also be measured prior to use.

(B) The entire plant should be harvested, rinsed with a minimum amount of water (which is returned to the nutrient solution), and separated into its respective organs as follows: carrot—root peels, peeled roots, and tops; cucumber—fruit, vines plus leaves, and roots; corn—kernels, husk plus cob, stalk plus leaves, and roots; lettuce—tops and roots; onion—bulb and tops; ryegrass—tops and roots; soybean—grain, chaff plus tops, and roots; oats—grain, chaff plus tops, and roots; tomato—fruit, vines, and roots; cabbage—head and roots. Plants from two pots in each treatment may be pooled, giving three replicate sample pools per treatment. After the fresh weights of the plant organs are obtained, each pool of organs should be subsampled for percent moisture determinations by drying, at 70 °C for 24 h in a forced-air drying oven, and weighing. Percent moisture determined from these subsamples is used to correct for dry weight of the fresh samples which should then be homogenized and extracted in organic and aqueous solvents. If radioisotopes are used, the amount of test chemical in each extract should be determined by liquid or solid scintillation depending on the type of radiation; otherwise, the amount of chemical should be determined by standard methods. At test completion,

the root support material should be washed in organic and then aqueous solvent and analyzed for test chemical before discarding.

(C) A suggested extraction procedure appropriate for many organic chemicals is as follows: Plant material (1g) should be homogenized with 1g of solvent-washed anhydrous sodium sulfate in 4 mL of hexane or acetonitrile. The homogenate should be filtered or centrifuged, the solid residue rinsed with an appropriate organic solvent, and the filtrate or supernatant combined with the rinse. The solid residue should be extracted by sequentially (*1*) homogenizing in water, (*2*) centrifuging and decanting the supernatant, (*3*) extracting of the pellet with 6N hydrochloric acid at 60 °C for 10 h, (*4*) subsequently digesting with 10N potassium hydroxide, and (*5*) combining supernatants. The resulting solution should be analyzed by liquid scintillation spectrometry or gas liquid chromatography (GLC) methodology. The organic extract should be evaporated under vacuum to a sufficiently small volume for thin layer chromatography (TLC) and co-chromatographed on silica gel plates with known standards of the parent chemical. If radioisotopes were used, the chromatographs could be scanned for radioactive substances on a radiochromatogram scanner. Alternatively, zones may be removed from the plates, extracted, and the quantity of chemical from each zone determined by liquid scintillation spectrometry or GLC methodology. The unextractable chemical in the remaining residue may be determined by oxidizing the residue in a complete combustion oxidizer.

(ii) **Gaseous test chemicals**. (A) A gas exposure system yields requisite data for a direct calculation of uptake rates. At steady state, chemical uptake may be determined by a mass balance calculation. Correction for adsorption to surfaces of the exposure chamber should be made by operating the system without plants. Pots filled with hydroponic solution or support media should be included in the sytem adsorption calibration. Consequently, chemical analysis of plant tissues exposed to gaseous chemicals may not be required in order to demonstrate and quantitate uptake rates.

(B) Altered rates of net photosynthesis, transpiration, and stomatal conductance are anticipated as a result of chemical uptake. Rates of these physiological processes before, during, and after exposure to the gaseous chemical should be determined. Data required for these calculations are available as a consequence of maintaining the specified environmental conditions within the fumigation chamber.

(iii) **Numerical**. Mass of pooled plant organs and pooled whole plants should be measured for the uptake and translocation test and subjected to chemical analysis (above) to quantify free parent test chemicals, its metabolites and soluable and bound residues. Mass balance of the test chemical and evapotranspiration rates of the plants are also determined. Means and standard deviations should be calculated and plotted for each

of the above for every treatment and control. The data should also be subjected to an analysis of variance.

(e) **Test conditions**—(1) **Test species.** (i) Test plants recommended for the uptake test include:

(A) *Lycopersicon esculentum* (tomato).

(B) *Cucumis sativus* (cucumber).

(C) *Lactuca sativa* (lettuce).

(D) *Glycine max* (soybean).

(E) *Brassica oleracea* (cabbage).

(F) *Avena sativa* (oat).

(G) *Lolium perenne* (perennial ryegrass).

(H) *Allium cepa* (common onion).

(I) *Daucus carota* (carrot).

(J) *Zea mays* (corn).

(ii) Other species of economic or ecologic importance to the region of impact, may also be appropriate and selected for testing. Two species of potentially differing sensitivity should be selected such as monocotyledonous and a dictyledonous species. It is further suggested that the test plants selected should be of different growth forms, e.g., a root crop and a leaf crop.

(2) **Facilities**—(i) **Apparatus.** Greenhouses, environmental chambers, or growth rooms should provide adequate environmental control to meet the carbon dioxide, humidity, irradiation, photoperiod, and temperature specifications. Chambers should be designed to prevent escape of internal air into the external environment other than through appropriate filtering material or media to prevent contamination of the external environment with radioactive and/or test substances. Laboratory facilities for plant extractions and chemical determinations should include nonporous floor covering, absorbent bench covering with non-porous backing, and adequate disposal facilities to accommodate plant nutrient, test, and wash solutions containing radioisotope and/or test chemical at the end of each run, and any bench covering, lab clothing, or other contaminated materials.

(ii) **Containers and support media.** For testing purposes, at least 24 polyethylene pots sufficently large to grow at least five plants up to 28 days or one to three plants to maturity are required. If plants are grown hydroponically, one plant per pot may be the preferred method. If a carrier control is needed, 30 pots are used. Potting containers used ine ach experi-

ment should be of equal size and volume and possess the same configuration. When sand or glass beads are used the potting containers should be filled to within 2.5 cm of their tops with sand or glass beads. Perlite, vermiculite, native soils, etc., should not be used for root support. Potting containers should be covered with opaque polyethylene bags to exclude light and minimize volatilization of test chemical.

(iii) **Cleaning and sterilization.** Potting containers, nutrient storage containers, and root support medium should be cleaned before use. All equipment should be washed according to good standard laboratory procedures to remove any residues remaining from manufacturing or use. A dichromate solution should not be used for cleaning beads or pots. Rooting media other than glass beads should be discarded at the end of the experiment. Disposal should conform to existing regulations.

(iv) **Nutrient media.** Half-strength modified Hoagland nutrient solution should be utilized as nutrient media for this test. Hydroponic solution should be aerated and sand or glass beads potting containers should be filled with nutrient solution and drained periodically. An automated system design is recommended.

(3) **Test parameters.** Environmental conditions should be maintained as specified below:

(i) Clean dioxide concentrations at 350 ± 50 ppm.

(ii) Relative humidity approaching 70 ± 5 percent during light periods and 90 percent during dark periods.

(iii) Irradiation, measured at 1 meter from the source, at 350 ± 50 $\mu E/m^2$ sec at 400 to 700nm.

(iv) Photoperiod of 16 h light and 8 h darkness for all species except soybean which should be provided with 11 h light and 13 h darkness prior to flowering.

(v) Day/night temperatures at 25/20 ± 3 °C.

(f) **Reporting.** Reporting requirements of 40 CFR Part 792—Good Laboratory Practice Standards apply to this guideline. Concentrations should be expressed in appropriate weight units per grams of dry plant material and of water lost by evapotranspiration. Data should also include initial and final total concentration of the test chemical in the growth media. These data will be used to compute mass balance. The following should be reported for each of the species tested in tabular form:

(1)**Solid and liquid test chemicals.** (i) Concentration of chemical in nutrient solution and root support material when chemical is soluble in water or solubilized with a carrier compound, as well as the concentration of carrier compound in nutrient solution when carrier is used, or the quan-

tity of chemical per unit weight of root support material when it is coated on the material.

(ii) The quantity of chemical, the concentration at which it was applied, and the number of applications for those chemicals applied to the foliage.

(iii) Environmental conditions (day/night temperatures, relative humidity, light intensity, carbon dioxide concentration, and photoperiod) and the occurrence and extent of any disruption of environmental control facilities.

(iv) Mass of each pool of plant organs and by summation, the mass of whole plants (dry weight after 24 h at 70 °C).

(v) Concentration of free parent test chemical, metabolites and soluble residues, and bound residues in pooled plant organs and pooled whole plants.

(vi) Mass balance of chemical.

(vii) Means evapotranspiration rate per plant.

(viii) Visible effects of chemical, if any, on the intact plants.

(ix) Analysis of variance, F-test, means, and standard deviation about the mean are calculated under paragraphs (f)(1)(iv), (f)(1)(v), (f)(1)(vi), and (f)(1)(vii) of this guideline.

(2) **Gaseous test chemicals.** (i) Concentration of gaseous test chemical at inflow and outflow ports.

(ii) Environmental conditions within gas exposure system (air temperature, dew point temperature or water vapor pressure of incoming and outgoing air streams, light intensity, air speed within chamber, carbon dioxide concentration at inflow and outflow ports, gas flow rate into and out of exposure system).

(iii) Mass (dry weight after 24 h at 70 °C) of leaves and stems and surface area (one side of leaves) in the exposure system.

(iv) Calculated measurements of photosynthesis, transpiration, and stomatal conductance before, during, and after exposure to test chemicals.

(v) Visible effects of chemical, if any, on the plants.

(vi) Analysis of variance, F-test, means, and standard deviation about the mean are calculated for each of the following:

(A) Steady state rates of photosynthesis, transpiration, and chemcial uptake before, during, and after fumigation.

(B) Stomatal conductance or leaf diffusion resistnace before, during, and after fumigation.

(vii) If uptake is determined by direct chemical analysis of plant tissues, then the reporting requirements also include:

(A) Concentration of free parent test chemical, metabolites and soluable residues, and bound residues in pooled plant organs and pooled whole plants.

(B) Mass balance of the chemical.

(C) Analysis of variance, F-test, means and standard deviation about the mean under paragraphs (f)(2)(vi)(A) and (f)(2)(vi)(B) of this guideline.